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Bioethics for Biotechnologists: From Dolly to CRISPR

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Abstract: Bioethics, as a discipline, has developed mainly, but not exclusively, around themes of moral importance for the medical practice, such as abortion and euthanasia, a never ending discussion that has been shaped by social mores and influenced by scientific and technological advance. However, in the past 20 years an important shift has been taking place, one where bioethical issues and their discussion are starting to being driven by the so-called emerging biotechnologies, from cloning to genome sequencing and editing. If Bioethics is concerned with human beings, and their interaction with other living beings and the environment, it makes sense for Biotechnology, by definition the use of living systems or organisms to develop products, to become an important, if not the most important, source of bioethical conflicts in modern era and for future society. As Biotechnology keeps expanding and becomes entangled in everyday life, so does the need for ethical competent biotechnologists, with competencies built not only on ethical principles but also on a realistic grasp of the impact these technologies could have on human society and the world we inhabit.

Keywords: Biotechnology, genome editing, animal cloning, ethics, fairness, biothreats, biosafety, biosecurity

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1 Introduction

Bioethics has evolved from a discipline mostly perceived as being focused on moral issues ranging from medical practice to a wider endeavor where concerns regarding the nature of human identity, animal welfare and the environment are becoming increasingly important, if not central. This is the result of the unrelenting pace at which the life sciences and biotechnology are advancing, defying old paradigms and creating new ones. The past 20 years have witnessed 3 milestones in the life sciences; the birth of the first cloned mammal, Dolly (Callaway 2016), the completion of the Human Genome Project (Venter et al. 2001) and the discovery and harnessing of CRISPR, a game changer in the field of genome editing (Mariscal and Petropanagos 2016).

The main goal of biotechnology is and has always been to improve people's lives; this is in line with ethical goals and is in itself an ethical vision. Animal domestication, agriculture and fermentation, all contributed to a better quality of life with the consequent expansion of human population through the planet. More recently, technological and medical advances such as nitrogen fixation, vaccines and antibiotics have contributed to a longer life expectancy and population explosion arising important issues, perhaps the most important the allocation of resources in a world edging close to ecological exhaustion. It is to be expected that many of the emerging biotechnologies of today will become an established part of daily life in the future. But will these changes be positive for society? Have the risks and benefits been analysed? All these changes often occur without consultation with the general population, and sometimes without ethical reflection on the part of the scientists and technologists involved (O'Mathúna 2007).

2 Biotechnology: the road to genetic engineering

The history of biotechnology can be roughly divided into three categories: Ancient Biotechnology, Classical

Biotechnology and Modern Biotechnology. While ancient biotechnology can be described as a series of developments, such as the domestication of plants and animals and sometimes accidental discoveries, like bread, cheese and wine, Classical Biotechnology lingers as a transitional period were the fundamental tenets of genetics and molecular biology were established. Modern Biotechnology officially started from the second half of the 20th century with the development of tools for the manipulation of nucleic acids, cloning and recombinant DNA technology (rDNA), critical for the development of genetic engineering (Verma et al. 2011). It is interesting to note that Bioethics and Modern Biotechnology were born around the same time and although there is discrepancy regarding the origin of the word "bioethics" there is agreement it was coined between 1970 and 1972 (Pellegrino 1999; Martensen 2001). In 1972, Paul Berg created the first recombinant DNA molecule; the next year Boyer and Cohen created the first genetically modified organism, these events marked the birth of genetic engineering as a force for technological advance (Giassetti 2013).

For almost three decades, up to 2012, recombinant DNA technology had proved to be a solid tool for genetic engineering. Despite limitations: time-consuming, lacks precision, low efficiency and high costs, it remained for many years the backbone of genetic engineering, along with DNA sequencing and the Polymerase Chain Reaction (PCR). Given the limitations of the technique, rDNA was better suited for single cell organisms and bacteria, and since the 1970's a vast array of medical and industrial applications have been developed: human insulin and growth hormone-producing recombinant bacteria being among the most important. Recombinant DNA technology also became a tool for research in laboratories around the world.

Genome editing as a new approach in genetic engineering started to develop recently and aim to manipulate the genome by adding, subtracting or replacing genes inducing site-specific breaks in the DNA using nucleases, enzymes able to cut DNA, and taking advantage of the natural DNA repair system to close the gap. Zinc-finger nucleases (ZFN) were discovered in 2002, followed by transcription activator-like effector nucleases (TALENs), which represented a significant advance in the ability to control the manipulation of DNA. However, despite the improvement in precision, both ZFN and TALENs still needed significant expertise and resources, limiting widespread use. This all changed with the discovery of CRISPR/Cas9, a natural system of bacterial defense against bacteriophages, reinvented as a precision tool for the editing of the genome (Maxmen 2015; Au 2015)

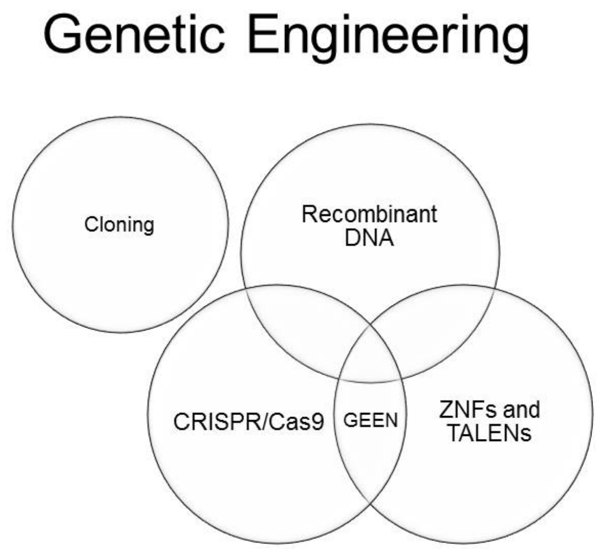

Figure 1: Genetic engineering (GE) involves the manipulation and/or modification of the genome of living beings, for 3 decades recombinant DNA remained the main tool of GE but recently, engineered endonucleases (GEEN) such as ZFNs and TALENs have proved to be a more precise and efficient tool, the discovery of the CRISPR/Cas9 system took this one step further, revolutionizing GE. To differentiate from recombinant DNA technology, the modification of genomes using endonucleases is known as genome editing. Despite not involving modification of the genome, cloning is considered by many a type of GE

CRISPR stands *for Clustered regularly interspaced short palindromic repeats*, short snippets of viral DNA inserted into a bacterial genome, acting as spacers and working as a template to synthesize a type of interference RNA, able to recognize specific points in the genome of an infecting virus to mark sites to be cut by nucleases (Gaj et al. 2013). Cas9 is the most recognized of these nucleases, but others, such as Cpf1, have been recently discovered, expanding the toolbox of genetic engineering. If recombinant DNA technology was a step forward, CRISPR could be described as a quantum leap for genetic engineering and for the emerging biotechnologies of transgenic plant and animal development, synthetic biology, nanotechnology, xenotransplantation, gene therapy and population control through gene drives. These so-called emerging biotechnologies represent the next stage in the development of Biotechnology one that now aspires not only to use living beings to develop products, but also to tailor organisms through genome editing or synthetic biology approaches, to obtain new products for human use and consumption. These technologies make the ethical training of students and professionals of biotechnology in general even more urgent (Rasmussen and Ebbesen 2014).

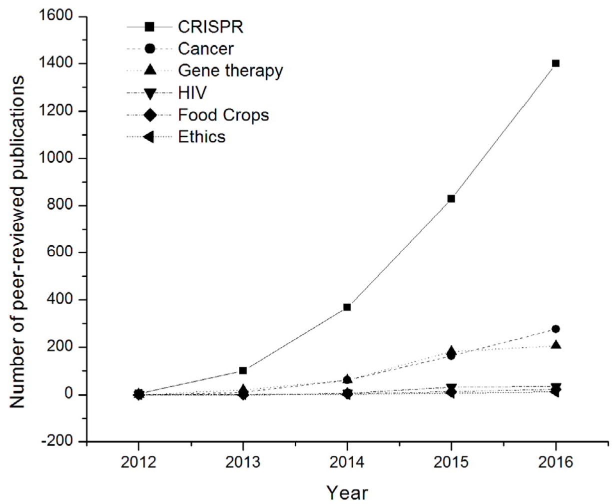

Figure 2: Since the discovery of the CRISPR/Cas9 system in 2012, genome editing has become an intense field of research; this is evident after reviewing the increment in publications involving this technology in recent years. The data shows the number of publications in 5 different fields where CRISPR/Cas9 was involved; research on disease being the most sought application of CRISPR/Cas9. The search was performed using PubMed, data was retrieved between November 28th and December 5 of 2016. Key words included CRISPR, and the combination of CRISPR plus cancer/gene therapy/HIV/crops or ethics

3 Emerging biotechnologies

Technology typically advances step by step, but occasional more rapid progress can be almost disruptive. All emerging technologies have one key feature in common despite being diverse in nature and purpose, they have potential to change the status quo. Other features are related to their stage of development, their novelty and potential risks. Emerging technologies include among others, artificial intelligence, 3D printing, robotics and a vast array of biotechnology disciplines. According to the Nuffield Council for Bioethics (Nco B 2012), there are a few key areas that can be identified as particularly significant: regenerative medicine, genomic medicine, synthetic biology and nanotechnologies, particularly nanomedicine. Besides their disruptive nature and potential, these emerging biotechnologies also pose unknown risks to humans and the environment, risks that need first to be assessed according to best ethical and biosafety and biosecurity guidelines.

4 Ethical reflection in Biotechnology

The four ethical principles are useful for the discussion of the many challenges brought by emerging biotechnologies, particularly DNA-based technologies. The first ethical principle is **beneficence**, a moral imperative to contribute to other people's well-being. To act on this principle demands a decision of what is good for others; it also requires respecting other people's autonomy, and honour individual requests. The principle of **non-maleficence** (*Primum non nocere*) asks us to not harm others; we should avoid needless harm or injury, by omission or action. **The principle of justice** is an essential requirement, it demands the fair treatment of individuals, fair access to treatment and distribution of resources regardless of social status, age, ethnic background, sexual orientation, disability, legal capacity and gender. This is what we mean by 'fairness'. **Autonomy** is based on the idea that humans are capable to direct their life in a rational way and have the right to do so; another way to express this principle is the ability to take decisions regarding one's well-being and fate without external influences (Lawrence 2007). In medical practice, where the well-being of patients is at stake, the four principles can be applied with relative unambiguity, in biotechnology, however, there are many instances where their use and value is not very clear and other considerations must be taken, such as risk analysis. Since there is a vast amount of literature addressing ethical issues surrounding designer babies, gene therapy, xenotransplants, synthetic biology and direct-to-consumer genetic testing, the aim of this paper is to bring attention to some issues where the use of the four principles may prove enlightening.

5 Animal Cloning

This year marks the 20th anniversary of the birth of Dolly, the cloned sheep, a landmark event for genetic engineering and the life sciences. Dolly lived a mere 6 years before being put down for health reasons but four of her sister clones are still alive and thriving (Sinclair et al. 2016). Early cloning success brought focus to bear on reproductive human cloning and the ethical issues regarding human identity, autonomy and rights, and there remains agreement that human cloning should not be pursued. This does not mean that reproductive cloning

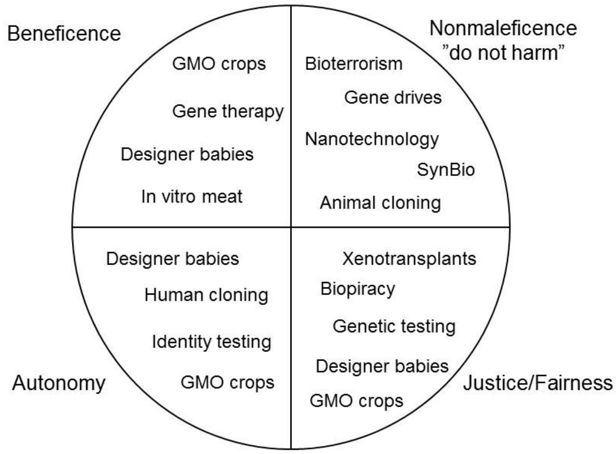

Figure 3: There are four ethical principles that may be used as guidelines to assess the ethical issues arising from biotechnology. In this figure, emerging biotechnologies were assigned to one of the four principles against which ethical issues have been identified (SynBio stands for Synthetic Biology)

has been abandoned as a scientific and business pursuit and at least 20 distinct animal species have been cloned to this day. There is a scene in the movie *The 6th Day* (Spottiswoode 2000), where Johnny Phoenix, a famous pro football player, is severely injured during a game and is taken to hospital. after a brief phone exchange a representative of the owners of Johnny's contract shuts down the machine keeping him alive. Johnny is then replaced by a clone of himself, in his world human cloning is illegal but is still being done, while animal cloning is acceptable and is profitable business. According to Vanity Fair's article *How Champion-Pony Clones Have Transformed the Game of Polo*, in Argentina, Crestview Genetics offers to produce exact genetic duplicates of an original animal; their business being oriented to the cloning of polo champion horses. Also in Argentina, a family paid between $60,000 and 100,000 USD to get a clone of their deceased pet, a first for the country. Cloning has gone commercial and there is a renewed optimism and interest in this technology. The fact that animals rather than humans are being cloned should not be dismissed as unimportant, as from an ethical point of view there are issues to be addressed. Cloning animals to win trophies, and perhaps money, raises issues of justice, not everybody is in a position to pay for such services and those with money have a greater chance at winning. Something similar can be said of cloning dead pets, only for those who can afford it, but most troubling is the idea of rejecting death as a part of the life experience. So the benefit of cloning pets seems to be more a human indulgence and is not a rational, ethical act.

6 Bioterrorism

Biosafety sets out measures to prevent the unintentional release or exposure to pathogenic or genetically modified organisms (GMO) whereas biosecurity measures are directed at preventing the intentional, unauthorized access or release of these entities (Kumar 2015). *Bioterrorism* is the use of pathogenic microorganisms or infected materials to cause terror and death in targeted populations. It has a long history, but it was after the terrorist anthrax attacks to the city of New York in 2001 that concerns around biological threats increased significantly. There is no ambiguity regarding the use of biotechnology for these aims, the principle of non-maleficence should be followed. The response to acts of bioterrorism is called *biodefense*, and it is a good example of a concept known as *dual-use*, according to which some technologies developed for military pursuits can be useful for civilian purposes. In this sense, a lot of research involving pathogenic microorganisms is performed in military facilities across the United States, justified by the search for preventive measures against biological warfare. However there are ethical issues concerning the diversion of government funds from public health, risks derived from facilities close to populated areas and the restriction on publication of the research performed in these facilities (King 2005). CRISPR technologies add another dimension to this problem. As a tool for genome editing; CRISPR is more efficient, cheaper and easier to master than previous genetic engineering tools, giving upsurge in numbers of *biohackers*, amateurs working outside official institutions, yet posing problems of biosafety and biosecurity since their activities escape regulation and government surveillance (Ledford 2015).

7 Biopiracy (theft of traditional knowledge)

The wealth of nations can be measured in terms of their biodiversity; those indigenous animals, plants; microorganisms which represent an important source of new compounds and biomaterials with potential to combat emerging diseases or to produce valuable goods. South America is perhaps the most rich in biodiversity, for example in Brazil, an emerging economy. This biodiversity is an asset coveted by the industrialized nations, giving rise to the upsurge of *bioprospecting*, the identification and commercialization of natural resources as bioproducts. However, there is a dark side to this activity, known as *biopiracy*, the illegal and unfair appropriation of the traditional knowledge or biomaterials of indigenous people without sharing the benefits (Efferth et al. 2016). This appropriation extends beyond traditional knowledge or biomaterials to the genetic information of natural resources, the germplasm, through the claiming of intellectual property rights, such as gene patents, by corporations based in developed countries. To address these issues, the Nagoya Protocols on Access and Benefit Sharing of Genetic Resources (ABS) was established in 2010. This protocol is part of the United Nations Convention on Biological Diversity, whose goals include the conservation of biological diversity and its sustainable use as well as the promotion of fair and equitable sharing of benefits arising from genetic resources. The Nagoya Protocols provide a series of rules on how nations access and share biodiversity, Biotechnologists should be aware of these issues and their legal and moral implications (Mackey and Liang 2012).

8 Gene drives

Gene drive systems seek to control population growth (Sinkins and Gould 2006). Such strategies have most benefited from the discovery of CRISPR and are the most controversial too. Gene drives involve the manipulation of the germline of species considered harmful to humans, usually pests or disease vectors. The aim is to spread deleterious traits or resistance genes for diseases among wild populations. The approach has been proved to be efficient; researchers at the University of California San Diego recently used the CRISPR/Cas9 system to engineer a gene drive to introduce two genes that confer resistance to malaria in the mosquito vector for the disease *Anopheles gambiae* (Gantz et al. 2015). Although the aim of such research seeks to benefit humans, the consequences of eradicating species from the ecological web are mostly unknown, making risk prediction a difficult task. Under the current state of knowledge, gene drives represent a threat to biodiversity and food security and there are also concerns regarding national sovereignty too. Consequently the Convention on Biological Diversity has been urged to call for a moratorium on the use of gene drives systems (Callaway 2016b). Other ethical issues worthy of reflection are related to our duties as a dominant species on the planet. For example, do we have a right to wipe other species out? Are these other species important beyond human needs and problems? This presents a problematic anthropocentric view of the beneficence principle.

9 Concluding Remarks

Biotechnologists are at the front line of a life sciences revolution that will ultimately shape the society of the future. DNA-based biotechnology has become central to technological advancement and progress. It is important then, for professionals in this discipline, to be aware of the potential consequences of their scientific endeavors on society and the individual. For the many challenges arising from basic or applied biotechnology, we can use the four principles of ethics for discussion and analysis. Taking them as a guideline, one could easily advice against the use of biotechnology tools for weapon development in bioterrorism, do not harm. However, for many other issues, resolution and governance may not come easily, this can be exemplified by the issues raised by the cloning of animals for profit or personal satisfaction. Regarding issues of justice, it is important to stress that while the primary concerns of the developed world are currently focused on human genome editing, these concerns, although valid for emerging economies too, overshadow other bioethical issues more immediate in their urgency, such as population control and biopiracy, where the imbalance of economic and political power plays a significant role. Biotechnology is growing at a fast pace in the developing world, hence, it is important to be aware and proficient to assess the impact of biotechnological advance in the light of local values and needs.

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